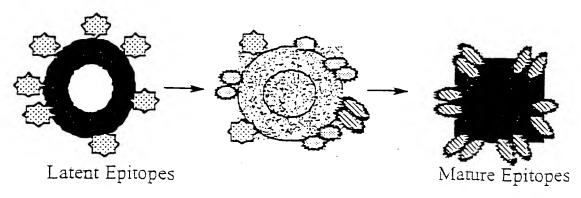
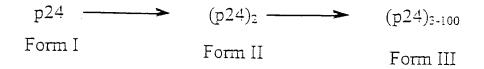
## FIG 1

Scheme 1. Conformational search and chemical conversion during rHBsAg maturation

A. Schematic Representation for Epitope Evolution

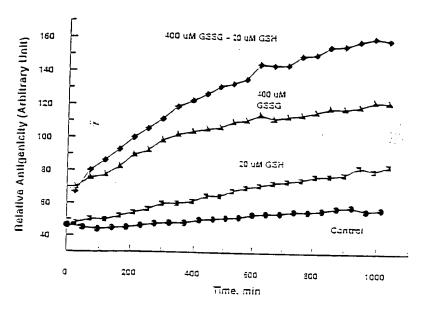


B. Chemical Conversion - Interchain Disulfide Bond Cross-linking

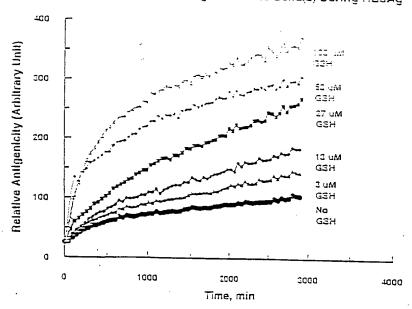


# FIC 2

Glutathione-mediated maturation of tHBsAg at 37 °C. Top: Synergistic effects of GSH and GSSG; Bottom: Better conformation of HBsAg can be achieved by higher concentration of GSH.



GSH-Catalyzed Unscrambling of Disulfide Bond(s) During HEsAg



# $V_{\mathbf{k}}$ gene region sequence comparisons

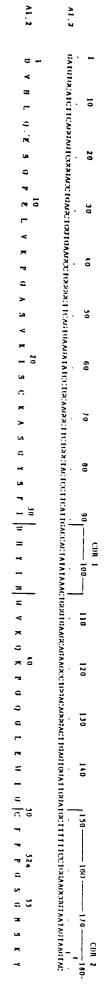
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CDR 2

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# $V_{\rm h}$ gene region sequence comparisons of anti-HBs



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### F16 4

### Flow Chart

### In Vitro Relative Potency Assav

DAY I

Pre-treat standard and samples - Section III.A.

Dispense 200 mcL of each treated standard or sample dilution into wells

Dispense 200 mcL of the Diluent Control and the Kit Controls into replicate wells

Add 50 mcL of conjugate to wells

Tap tray gently to mix

Add one bead to each test well

Incubate trays for 12-20 hours at 20-28°C

DAY 2

Wash beads 1 cycle with  $14 \pm 3$  mL distilled water

Transfer beads to tubes

Add 300 mcL of OPD substrate solution

Incubate 30 to 35 minutes at 20-28  $^{\circ}$ C in the dark

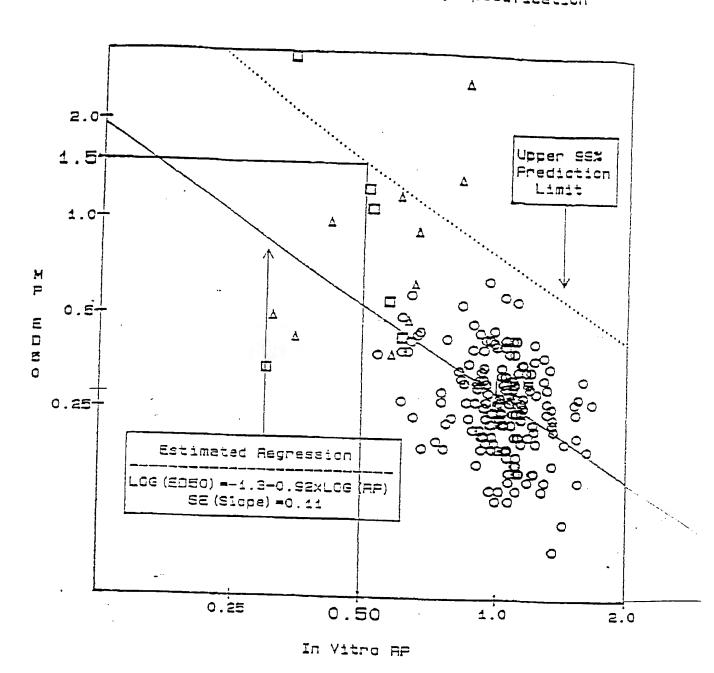
Add 1 mL of 1N Sulfuric acid to each tube

Read tubes in Quantumatic $^{TM}$  within 2 hours

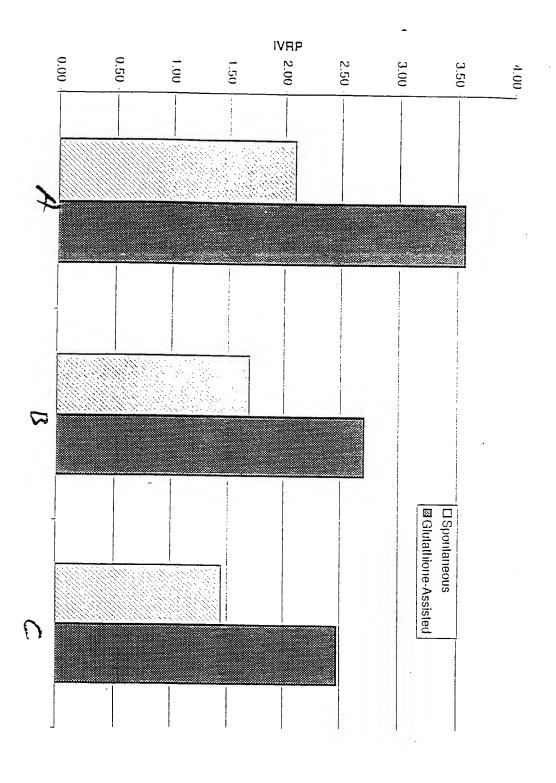
- Read Kit Controls blanked on substrate.
- Read Standards and test blanked on sample diluent.

F/6 5

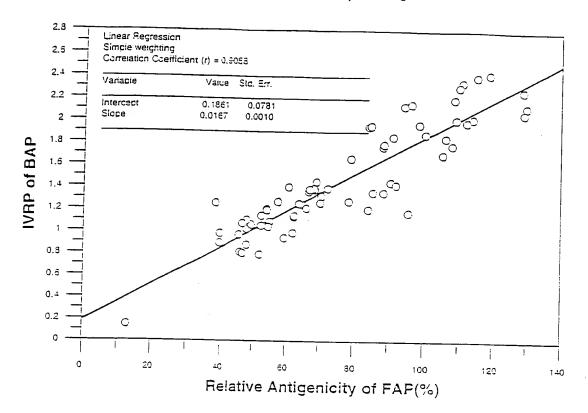
In Vitro Relative Potency Specification Corresponding to Mouse Potency Specification



Spontaneous vs. Glutathione-assisted Conformational Search During rHBsAg Formulation



### F167



Linear relationship between IVSP of BAP vs. relative antigenicity of FAP (%) by BIAcore.